Evaluation of a Factor Exclusion Experiment Designed to Assist in Elucidating the Etiology of Citrus Blight

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ABSTRACT. In 1986, 42 Delta Valencia trees on rough lemon rootstocks were planted at random in a 25-yr-old Valencia orchard affected by citrus blight. The trees were planted on sites from which blight affected trees had been removed. Five chemicals were used to treat 24 of the experimental trees. The chemicals used were Temik®, Rugby®, Benlate®, Ridomil® and Azodrin®. The other treatments included trees planted in holes (3 m × 3 m × 1 m deep) filled with virgin soil; trees planted in insect proof cages; and untreated controls. Based on zinc analyses, canopy rating and water uptake, only two trees have been identified with apparent blight. Serological assays conducted in 1997, 1998 and 2001 were, however, negative for all trees. There appears to be no relationship between the type of treatment and incidence of blight. The low incidence of blight may be as a result of the physiological changes brought about by severe pruning of the scions and root systems following relocation, owing to a severe drought.

Index words. Citrus blight, factor exclusion, etiology.

In South Africa, a blight-like decline was first reported in 1979 (4). Citrus blight (CB) occurs in more temperate areas of South Africa, but is a serious limiting factor in production in the hot, humid Letstitele area of Limpopo Province in the northern part of the country, which is the major Valencia production area of the industry (8). In South Africa, it has been determined that the rate of spread of CB is linear (12). This is typical of many abiotic diseases but the demonstration that the full complement of characteristic symptoms can be reproduced by root-graft inoculation, suggests that the disease is caused by a systemic infectious agent (7). This has been demonstrated repeatedly in Brazil, Florida and South Africa (11, 13). Several researchers have suggested that CB is spread by an aerial vector (1) or agents in the soil such as *Fusarium solani* and nematodes (3). The object of this experiment was to determine whether the incidence of CB in orchards of high disease pressure, could be reduced by supplemental applications of chemicals effective in controlling aerial vectors and soil-borne organisms, which are prevalent in South African citrus soils.

The experimental site selected for this experiment was a mature Valencia on rough lemon rootstock orchard approximately 25 yr old and which had a high incidence (25%) of CB. Forty-two 30-mo old Delta Valencia trees were planted randomly throughout the CB affected orchard during December 1986 on sites from which blighted trees had been removed. The trees were treated as follows:

i) Temik® (aldicarb) (30g/m^2) at 8 weekly intervals, alternated with Rugby® (cadusafos) for nematode control (Rugby® was alternated with Temik® to prolong the nematicidal effect of the latter, which is prone to accelerated microbial degradation (10),

ii) Ridomil® (40g/m^2) at 8 weekly intervals, for control of Phytophthora root rot,

iiii) Temik®/Rugby® plus Ridomil® (as above) at eight weekly intervals.

iv) Trees planted in insect proof cages (4 m × 4 m × 4 m) and treated with Azodrin® and Temik® at eight weekly intervals, for control of aerial vectors. Azodrin® concentrate was applied as a trunk paint application as previously described by Buitendag (2).

v) Soil drench with Benlate® (benomyl) (1g/L) at 8 weekly intervals, for control of *Fusarium solani*.

vi) Trees planted in holes (3 m × 3 m × 1 m deep) lined with two layers of 150 µ black polythene sheeting and filled with virgin soil, to exclude soilborne organisms and contact with remnants of CB affected roots, left after removal of CB affected trees.

The diagnosis of CB was based on canopy symptoms rated on a scale of 0-3; 0 = healthy; 1 = mild symptoms, 2 = moderate, 3 = severe; zinc concentration in the trunk wood (15); water uptake by the syringe injection method (9) and serological assays (5, 6).

The entire experiment was relocated to another CB affected orchard during July 1993, 7 yr after its initiation, owing to a severe drought. The trees were "stag-horned" and the root systems pruned back severely. The trees were replanted in holes 2m × 2m × 1m deep on sites from which CB affected trees had been removed. The insect proof cages were not replaced, but the trees originally planted within these cages were treated with Temik and Azodrin at six weekly intervals to reduce aphid populations and simulate insect-proof conditions as far as possible.

During the period 1987-1992 the trees planted in virgin soil and those in insect proof cages exhibited substantially better growth than trees in the other treatments. The trees in the cages exhibiting significantly superior growth, probably owing to the shading effect of the screen, and shade cloth on the top of the cages. During 1997 there was a shift in this pattern, although not significantly so, to the Temik plus Ridomil treatment, taking second place to the trees planted in virgin soil. The trees from insect proof cages remained significantly different from all the other treatments. There was no significant difference between the canopy volumes of the untreated controls and all the other treatments, with the exception of the trees in the insect proof cages, throughout the experiment.

Zinc analyses indicated that CB was absent from the experimental trees, the values were typical of healthy trees in mature orchards.

There were no significant differences in water uptake between the different treatments. None of the trees measured in 1997 exhibited restricted water uptake typical of trees affected with CB.

The mean visual canopy ratings of the trees in insect proof cages and virgin soil, as assessed in 1992, differed significantly from the other treatments, there were however no significant differences in the mean canopy ratings in 2001. None of the trees exhibited small fruit, delayed bloom or blotchy mottle foliar symptoms, typical of CB, the majority of the symptoms were twig die-back, sparse canopies and slight zinc deficiency symptoms. No signs of sprout development were observed on scaffold branches or at the bud unions of these trees. It is possible that the deficiency symptoms found in individual trees during 1992, were induced by *Citrus tristeza virus* stem pitting in the rootstocks of these trees (8).

Dot blot immunoassays conducted in 1998, 1999 and 2001, were all confirmed using Western Blots; none of the experimental trees assayed positive for the 12 kDa blight-associated protein.

Based on the four diagnostic markers used to diagnose for CB, none of the trees in the above experiment can be declared CB positive. Taken at face value many of the trees would be categorized as positive for CB for the sole reason that they exhibit three out of four of the diagnostic characteristics used for positive diagnosis.

In conclusion it may be stated that based on the above results none of the treatments were responsible for the low incidence of CB, but that it is probable that the severe root

pruning and pruning of the scion before transplanting the trees, resulted in reduced vigor and fruit production of the trees. According to Tucker et al. (14), increased vigor and induction of earlier fruit production in young trees resulting from excessive fertilization and irrigation, may contribute to the earlier expression of CB. The physiological changes brought about within the experimental trees by the "stag-horning" and root pruning procedure, resulting in light crops being produced, may have affected the development of CB in these trees.

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